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A new species of *Stigmidium* (*Mycosphaerellaceae*) on *Aspicilia* from North America

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Abstract — *Stigmidium lendemeri* is described from North America on probably five species of *Aspicilia*. The new species belongs to the *Stigmidium placynthii* group in *Stigmidium* s. lato, distinguished from *Stigmidium* s. str. by long 3-to-5 celled periphysoids.

Key words — lichenicolous fungi, pseudoparaphyses, taxonomy

Introduction

Stigmidium Trevis. 1860 is a genus of predominately lichenicolous taxa comprising over ninety binominals (Mycobank 2012; Knudsen & Kocourková 2010; Kocourková & Knudsen 2009a; Pérez-Ortega et al. 2010) and currently included in *Mycosphaerellaceae* (Lumbsch & Huhndorf 2010). The type species is *S. schaereri* (A. Massal.) Trevis., a lichenicolous species occurring on sterile thalli of *Solorina* species (Roux & Triebel 1994; Crous et al. 2007). *Stigmidium* is heterogeneous (Calatayud & Triebel 2003; Kocourková & Knudsen 2009b). *Stigmidium* s. str. has perithecioid ascomata with external periphyses sensu Roux & Triebel 1994, short 2-celled periphysoids (pseudoparaphyses "Type a" sensu Roux & Triebel 1994), and 1-septate ascospores and lacks interascal filaments (Roux & Triebel 1994; Kocourková & Knudsen 2009b; Pérez-Ortega et al. 2010). *Stigmidium* s. str. comprises at least 24 species (Knudsen & Kocourková 2010; Etayo & Osorio 2004; Pérez-Ortega et al. 2010).

A distinct group in *Stigmidium* differs from *Stigmidium* s. str. in having long periphysoids of 3–5 cells (pseudoparaphyses "Type b" sensu Roux & Triebel 1994), no interascal filaments, and more or less well-developed external periphyses (Roux & Triebel 1994; Kocourková & Knudsen 2009b). This group currently comprises five species: *S. clauzadei* Cl. Roux & Nav.-Ros.

(Roux & Navarro-Rosinés 1994), *S. epistigmellum* (Nyl. ex Vouaux) Kocourk. & K. Knudsen (Kocourková & Knudsen 2009a), *S. hesperium* Kocourk. et al. (Kocourková & Knudsen 2009b), *S. placynthii* Cl. Roux & Nav.-Ros. (Roux & Triebel 1994), and *S. squamariae* (B. de Lesd.) Cl. Roux & Triebel (Roux & Triebel 1994, 2005). The long periphysoids are of diagnostic value but often hard to observe, apparently breaking off and dissolving when asci fill the ascomatal cavity in mature ascomata. Practically speaking, species with long periphysoids can be keyed out by host and ascospore size after they have been described.

In this paper we describe a sixth species of *Stigmidium* s. lato with long periphysoids from North America and found on several *Aspicilia* species.

Materials & methods

Specimens were supplied by the Cryptogamic Herbarium of the New York Botanical Garden (NY) and the Lichen Herbarium of the University of California at Riverside (UCR). Specimens have been examined using standard microscopical techniques. Hand-made sections were studied in water and 10% KOH [K] and lactophenol cotton blue [LPB]. Amyloid reactions were tested in Lugol's iodine 1% with and without pre-treatment with 5% KOH [K/I]. Metachromatic reactions were tested with 1% solution of brilliant cresyl blue [BCr]. Ascospore measurements were made in water with accuracy of 0.5 µm and given in the form "(minimum–) mean minus standard deviation–**mean**–mean plus standard deviation (–maximum)" and followed by the number of measurements (n); the length/breadth ratio of ascospore is indicated as l/b and given in the same form.

Macro and microphotographs were taken with a digital camera Olympus DP72 on Olympus SZX 7 Stereomicroscope and Olympus BX 51 fitted with a Nomarski differential interference contrast.

The species

Stigmidium lendemeri Kocourk. & K. Knudsen, **sp. nov.** Plate 1 & 2

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Differs from other members of the *Stigmidium placynthii* group by its larger ascospores and its *Aspicilia* hosts.

Type: U.S.A. Pennsylvania. Lackawanna Co., Moosic Mountains, Montage Heath Barrens, sandstone and conglomerate outcrops on west slope, 41°21′05″N 75°40′18″W, 396–426 m, on *Aspicilia* species, Sept. 30, 2005, Lendemer 5544 w/ Schuyler (NY, **holotype**).

Etymology: The species is named for the North American lichenologist James C. Lendemer (NY), who brought the taxon to our attention, in honor of his work on the lichen biota of North America, on *Lepraria*, and on the journal Opuscula Philolichenum. We value him as both a colleague and good friend.

Infection not visible. Mycelial hyphae not observed. Ascomata perithecioid, black, globose to subglobose, ostiolate, 60–100(–120) µm high and 60–100(–120) µm wide, partially immersed in the host areoles, usually dispersed, one

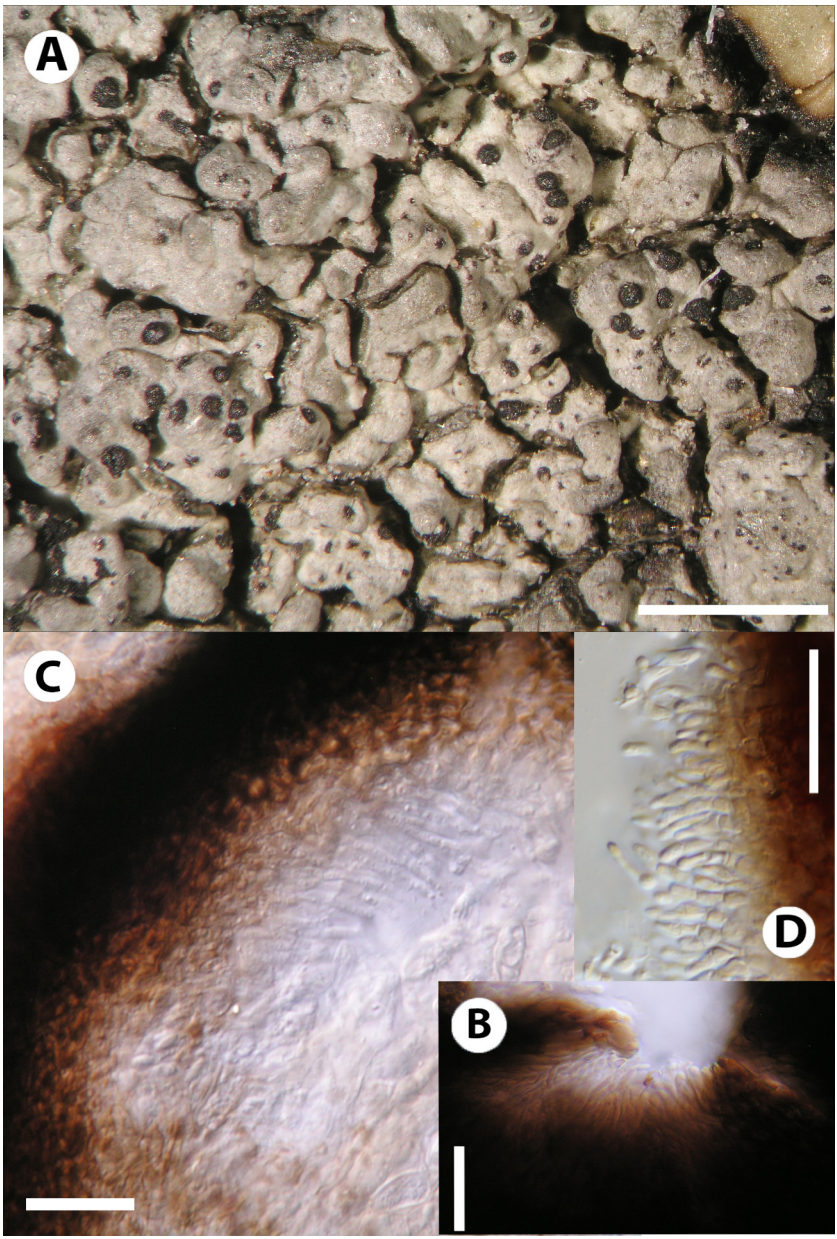


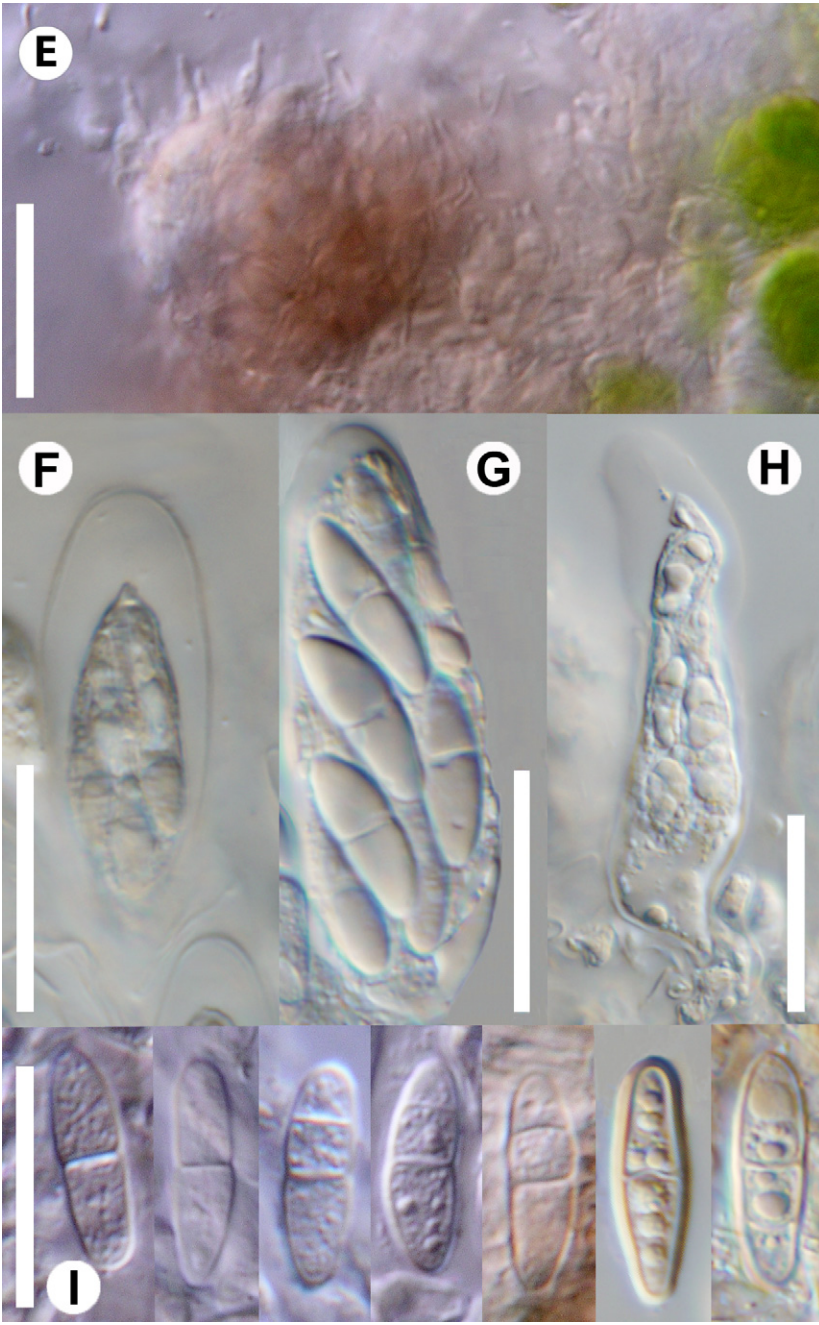
Plate 1. Figs A–D. *Stigmidium lendemeri* (Lendemer 5544, holotype). A, Infected thallus of *Aspicilia* sp. B, Ostiole with fringe of external periphyses. C, D Ascomatal section with pendent periphyses (pseudoparaphyses "Type b"). Scale bars: A = 1 mm; B–D = 20 µm.

or two per areole, causing no visible sign of infection but infected areoles not usually producing apothecia. Exciple dark brownish-black in upper 1/5–1/3, lighter brown or reddish brown in lower 4/5–2/3, but still dark even in thin sections, never hyaline, 10–15 µm thick in optical section, often thickest (up to 15 µm) in ostiolar area, of 2–4 compressed cell layers, textura angularis, cells mostly 4–9 × 3.5–4 µm in optical section, I–, BCr–. External periphyses visible as a dense fringe when viewed from above in mature ascomata, derived from ascomatal wall, 8–14 µm long, brown at base to pale yellowish-brown or almost hyaline at tip, mostly 1–1.5 µm wide, septate, cells 3–5 µm long, comprised of 2 to 3 cells, the base cell usually widest. Hamathecium of pendant periphysoids (pseudoparaphyses "Type b") originating from the upper wall of ascomatal cavity, hyaline, rarely branching, 12–15(–20) µm long, mostly 2 µm wide, septate, formed of 3 or more cells, cells 3–5 µm long, uneven in length. Lacking interascal filaments. Hymenial gel I–, K/I–, BCr+ light violet. Asci originating from lower wall of ascomatal cavity, fissitunicate, narrowly saccate, endoascus thickened in upper 1/3, sessile to stipitate, 8-spored, with ascospores distichously arranged, 20–30 × 10–12(–15) µm, I–, ascoplasma I+ red, exoascus BCr–, endoascus BCr+ blue-violet. Ascospores 1–septate, hyaline, brownish when over-mature and with 1 pseudoseptum (possibly also pseudotetrablastic but not seen), non-halonate, not constricted at septa, when 2-celled, cells usually equal in length, (14.0–)15.5–**17.3**–19.0(–21.0) × (4.0–)5.0–**5.8**–6.5(–7.0) µm (n = 40), l/b = (2.3–)2.6–**3.0**–3.4(–4.5); epispore BCr+ dark blue; perispore BCr+ very pale violet. Conidiomata 30–45 µm in diam., conidiogenous cells ampuliform 4.5–5.0 high, 4.0–4.5 µm wide, conidia cylindrical, strait or slightly curved, 4.5–6.0 × 1.0–1.5 µm.

Substrate and ecology — On probably five different *Aspicilia* species with stictic or norstictic acid or lacking extrolites that typically occur on non-calcareous rocks in open habitats (talus slopes, glades, rock ledges) with constant high humidity and on boulders in or near rivers or streams. We are unsure of the exact *Aspicilia* species in many cases as the genus is in need of revision. In a few cases the lack of conidia precluded accurate identification of the host.

Distribution — North America (California, Missouri, Ohio, Pennsylvania). Because it is host specific on genus level on multiple species we expect more reports from throughout North America. A taxon with similar ascospores causing a black infection was seen from California (UCR) and Kansas (NY) and needs further study.

Plate 2. Figs E–I. *Stigmidium lendemeri* (Lendemer 5544, holotype). E. Pycnidium, conidiogenous cells and conidia. F. Young ascus with apical typically short beak. G. Ascus with ascospores. H. Ascus releasing content. I. Nearly mature and mature ascospores. Scale bars: 20 µm.


E
F
G
H
I

Selected specimens examined — **U.S.A. California.** Mariposa Co., Sierra Nevada Mountains, Yosemite National Park, Merced River near Highway 140, 37°40′19″N 119°47′32″W, 653 m, on *Aspicilia* species (no extrolites) on boulders in river, Sept. 20, 2009, Knudsen 11680 (UCR); Tuolumne Co., Sierra Nevada Mountains, Yosemite National Park, Inspiration Point along Hetch Hetchy Road, Poopanaut, 37°54′12″N 119°50′01″W, 1601 m, on *Aspicilia* species (no extrolites), Sept. 19, 2009, Oregon State University Survey, Knudsen 11556 (UCR); **Missouri.** Iron Co., Ketcherside Mountain Conservation Area, Royal Gorge Nature Area, Big Creek, 37°32′21″N 90°41′01″W, on sterile *Aspicilia* species (stictic acid), March 26, 2006, Buck 49747 (UCR); Shannon Co., Ozark National Scenic Riverways, Rocky Creek, mesic forest, 37°07′N 91°12′W, 200–215 m, on *Aspicilia* species (norstictic acid) on rhyolite, April 16, 1997, Buck 31872 (NY); Wayne Co., Sam A. Baker State Park, Mudlick Mountains, 37°15′27″N 90°31′23″W, 300 m, on *Aspicilia* species (stictic acid), Oct. 15, 2003, Buck 45324 (NY); Washington Co., Mark Twain National Forest, Little Pilot Knob, 37°58′57″N 90°58′40″W, on *Aspicilia* species (stictic acid), May 24, 2003, Harris 47935 (NY); **Ohio**. Scioto Co., along Pond Lick Run, Shawnee State Forest, sandstone outcrops along stream in oak woodland, 38°42′20″N 83°08′25″W, 198–213m, on *Aspicilia* species, May 21, 2006, Lendemer 7175 (NY); **Pennsylvania.** Bedford Co., Pleasant Valley, Buchanan State Forest, west-facing slope with granite and sandstone boulders, 40°03′18″N 78°30′43″W, 518–548 m, on sandstone, on *Aspicilia* species, May 18, 2006, Lendemer 7294 (NY); Berks Co., west slope of Mt. Pleasant, French Creek State Park, 40°11′33″N 75°47′10″W, 213–243 m, on *Aspicilia* species, Nov. 19, 2006, Lendemer 8036 (NY); Bucks Co., Ringing Rock County Park, diabase boulder field along stream, 40°33′43″N 75°07′42″W, 137–152 m, on *Aspicilia* species, Sept. 15, 2005, Lendemer 4982 w/ Schulyer (NY); Carbon Canyon, east shore of Leigh River, Leigh Gorge State Park, cliff along railroad track, 40°57′58″N 75°42′22″W, 304 m, on cliff, on *Aspicilia* cf. *laevata*, Nov. 9, 2003, Lendemer 1491 w/ Rhoads (NY).

Discussion

The presence of long periphysoids with 3 or more cells places *Stigmidium lendemeri* in the *S. placynthii* group (Roux & Triebel 1994) in which we currently recognize five other species. "The small differences in ascospores size are assumed to be an important morphological expression of phylogenetic distance." (Kocourková & Knudsen 2009b). These small differences (compared with *S. lendemeri*) are summarized below, but in addition all differ in the host genera and species infected.

(1) *Stigmidium placynthii* on *Placynthium nigrum* has smaller halonate ascospores, (7.5–)9.5–**11.1**–12(–13.5) × 3.0–**3.3**–3.5(–4.0) µm.

(2) *Stigmidium epistigmellum* on maritime *Caloplaca* species has narrower ascospores, (14.5–)15.8–**17.5**–19.1(–21.5) × (3.5–)3.9–**4.2**–4.7(–5.0) µm.

(3) *Stigmidium squamariae* on several *Lecanora* and *Rhizoplaca* species has shorter halonate and constantly hyaline ascospores, (8.5–)9–**10.9**–12.5(–14) × (4–)5–**5.3**–5.5(–6) µm, and lacks reactions with BCr.

(4) *Stigmidium clauzadei* on *Verrucaria nigrescens* and *V. viridula* has generally shorter and wider 1-septate hyaline and halonate

ascospores, (10)–12.5–**15.1**–19 × (4.5)5–**5.5**–6.5(–7.5) µm, and all parts BCr– except perispore BCr+ pale blue.

(5) *Stigmidium hesperium* on *Caloplaca* species has shorter and narrower ascospores, (12.5–)13.0–**13.75**–14.5(–15.5) × (3.5–)4.0–**4.5**–5.0(–5.5) µm.

The only *Stigmidium* previously reported on *Aspicilia* is *Stigmidium aggregatum* [= *Pharcidia aspiciliae*] on *Circinaria calcarea* [≡ *Aspicilia calcarea*], which has much larger ascospores, 22–30 × 4.5–9 µm and is apparently known only from two lost types (Kocourková & Knudsen 2010). Based on our study of the problem, *S. aggregatum* may not even be a *Stigmidium*.

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